Chapter 2

REVIEW OF LITERATURE
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2.1. Genetic base of *Hevea*

The genetic base of *Hevea* is narrow (Wycherley, 1968; Schultes, 1977; Allen, 1984). From this little genetic foundation spectacular yield improvement of about ten times have been achieved (Varghese, 1992). However, reports of Tan, (1987); Ong and Tan, (1987); Simmonds, (1989) elucidate that the genetic advance gained in the early breeding phases seems to have slowed down in the more recent phases of breeding. There are a number of factors that narrow down the genetic variation, viz., (1) wider adaptation of clonal propagation by budding (2) directional selection for yield (3) and cyclical assortative breeding pattern. Such unidirectional selection for yield over the years ignoring the genetic variability with regard to secondary characters (Wycherley, 1969) has reduced the genetic variability in the population. In generation wise assortative mating best genotype in one generation is used as the parents for next and so on (Simmonds 1986a, 1989). So the parentage of the best clones can be traced back to a limited number of parent genotypes (Tan, 1987 and Varghese, 1992). However, efforts are in progress to broaden the genetic base of *Hevea* by the introduction of wild germplasm from the centre of origin and introduction of exotic clones. Moreover, the highly hetrozygous nature of the clone can also be exploited for enhancing the existing genetic variability.

Hybridization programme between clones in India started in 1954 with the available clones introduced earlier into the country (Nair and Panikkar, 1966). So far
around 5700 hybrid seedlings have been produced and about 1500 clones were developed (Varghese et al., 1990) of which RRII 100 series (Nair and Panikkar, 1966; Nair and George, 1969; George et al., 1980 and Nazee et al., 1986), 200 series (Saraswathyamma et al., 1980) and 300 series (Premakumar et al., 1984) are of commercial importance. Among the initial selections designated as RRII 100 series of clones (Nair and George, 1969) RRII 105 is the most outstanding clone. Hybridisation and ortet selection are the two conventional breeding methods practiced in Hevea that contributed to substantial increase in productivity. The identification of variability and successful implementation of bud grafting are the two developments that successfully led to the synthesis of early primary clones through ortet selection or mother tree selection.

2.2. Major yield components

2.2.1. Growth

Growth is one of the most important characteristics of a clone, next to yield. During the first few years, growth is mainly in length, and a rapid increase in girth becomes noticeable after the trees are a few years old. Different clones have different characteristic growth patterns (Polhamus, 1962). Girth measurements are considered an important criterion of tappability, since vigorous clones enable early opening of the trees for tapping (Ostendorf, 1932; Vollema and Dijkman, 1939; Dijkman, 1951; Peries, 1970; Webster and Paardekooper, 1989; Wright, 1998). Annual growth pattern of rubber trees during the immature and mature phases have been discussed in detail (Templeton, 1968, 1969; Pillay, 1980; Webster and Paardekooper, 1989; Sethuraj and Mathew, 1992; George and Jacob, 2000) and the relationship between girth increment rate and rubber yield in different clones have been well established. (Nga and Subramanian, 1974; Ong, 1981; Chandrasekar, 1994 and Tuy, 1997). In general, a growth retardation is observed during tapping, which also shows wide clonal variation.
Vollema (1941) reported that tapping does retard the growth of the *Hevea*. However, Schweizer (1941) indicated that rubber production in connection with growth increases. Annual girth increment showed difference among clones and high yielding clones generally showed low girth increment on tapping (Markose, 1984). However, Licy (1997) reported no such trend in a set of clones.

Analysis of monthly growth pattern and its duration in 13 *Hevea* clones from a traditional rubber growing tract in India, indicated that the peak growth period is about 2 months (Jul - Aug) and the active growth period is about 6 months (May - Oct.) (Chandrasekar *et al.*, 2002). Clonal differences in growth could be mainly attributed to genotypic differences. However growth is also determined by other factors like soil, climate, planting density, cultural operation etc. A few reports are also available on the monthly growth pattern of *Hevea* trees exposed to long drought and high summer temperature in subhumid tropies (Chandrasekar *et al.*, 1996; Chandrashekhar *et al.*, 1998). The results showed that by analysing the growth of rubber, potentially drought tolerant clones can be identified.

### 2.2.3. Physiological components of yield

Harvesting of *Hevea* is carried out by the controlled wounding of the bark of the tree trunk. This process is known as tapping. The standard girth generally accepted for commencement of tapping is 50 cm at a height of 125 cm from bud union for all the bud-grafted materials.

Sethuraj (1968, 1981, 1977) identified initial flow rate, plugging index, dry rubber content and length of the tapping cut as major physiological yield components in *Hevea*, and established the relationship between yield with its major yield components. The
influence of initial flow rate on yield has been established (Sethuraj et al., 1974; Yeang and Paranjothy, 1982). Sethuraj et al., (1974), Sethuraj (1981) found initial flow rate to be a clonal character. Relationship between seasonal variation in initial flow rate and variation in yield in different clones of Hevea has been reported (Saraswathyamma and Sethuraj, 1975).

Plugging index is an index that measures the extent of time during which latex flows out or it indicates the intensity of flow restriction mechanism operating in the latex vessel after tapping (Milford et al., 1969). It is also a major component of yield in Hevea. Clonal variation in plugging index have been established by Milford et al., (1969); Paardekooper and Somosorn (1969); Sethuraj et al., (1974); Saraswathyamma and Sethuraj, (1975). Yield has been found to be positively correlated to the initial flow rate (Paardekooper and Somosorn, 1969) and negatively correlated to plugging index (Milford et al., 1969).

The dry rubber content (DRC) of natural rubber latex, as obtained from the tree, varies from 30 to 40% by weight. Clonal characteristics, age of tree, length of tapping cut, frequency of tapping, stimulant application, time of tapping, environmental conditions etc. are some of the factors that affected DRC of latex (Kang and Hasim, 1982). Seasonal variation in yield is a result of volume of latex and not that of rubber content. Paardekooper and Sookmook, (1969) have established a negative correlation between yield and rubber content, this indicate that high yielding character of a clone is a result of the low plugging and that because of the higher extraction of latex the rubber content can be maintained only at a lower level under a given regenerative capacity (Sethuraj, 1992). The dry rubber content (DRC) of latex varies in different clonal lattices (Ng et al., 1979). It is reported that the rubber content of latex tends to become higher during the lowest yield periods like summer. Generally DRC is highest at the time of first opening and gradually reaches a stable condition.
Wittshire (1934) reviewed the earlier work on DRC variations. Schweizer (1936) noted a decrease in DRC during wintering which rapidly regained normal condition. Rebaillier (1972) also reported seasonal changes in DRC.

The length of tapping cut is another major component that control yield. The length of the tapping cut is determined by the girth of the tree for a given system of tapping (Sethuraj, 1992). pH of latex is considered to be an important factor regulating the metabolic activity of the laticiferous system and highly significant positive correlations have been obtained between pH and latex production under certain conditions (Brozozowska - Hanower et al., 1979; Cretin et al., 1980; Eschback et al., 1984).

The factors that influence annual rubber yield from a unit area is determined by the average yield tree¹ tap¹, the number of trees and number of tapping per year (Sethuraj, 1992). In India, the period of peak yield is from September to January (Sethuraj, 1992). Seasonal variations in yield is being mediated through variation in both initial flow rate and plugging index (Milford et al., 1969; Paardekooper and Somosorn, 1969; Saraswathyamma and Sethuraj, 1975 and Sethuraj, 1977). It was clearly demonstrated that the effect of drought and high temperature is mediated mainly through changes in plugging index than through changes in initial flow rate (Ninane, 1970).

The lowest value for rubber content is during monsoon season and highest values during dry seasons (drought season), which are the highest and lowest yielding periods respectively (Sethuraj, 1992). Negative relations between yield recorded over a period of one year and rubber content has been established (Heuser and Holder, 1931; Wittshire, 1934; Brozozowska - Hanower et al., 1979).
It is evident that the annual yield of a clone or clones depends upon the average values of initial flow rate, plugging index and dry rubber content through different seasons of the year. The environmental factors leading to lower flow rate and higher plugging will reduce yield. The variation in rubber content is related to its interaction with plugging and the volume of the latex lost. When yield is reduced due to high plugging rubber content will increases (Sethuraj, 1992). The latex yield is dependent on the rate and duration of latex flow (Markose, 1984).

2.2.4. Anatomical components of yield

The major type of laticifers exploited commercially for its latex is secondary laticifers distributed in the bark of tree trunk. Bryce and Campbell (1917) have briefly illustrated the structure of Hevea bark. The outermost protective layers of tissues are called cork cells. Interior to the cork cells, there are two more distinguishable zones, an inner soft zone and an outer hard zone. Sclerified stone cells are present in the outer zone, which makes this region so harder. The latex vessels in Hevea appear as concentric rings, alternating with layers of phloem cells. The differentiation of laticifers from the cambial cells is a rhythmic process and a ring of laticifers is produced each time (Premakumari et al., 1992). Latex vessels are developed by the activity of vascular cambium. Rao (1975), Premakumari et al., (1981) reported that the rate of cambial activity shows seasonal variations. Thomas et al., (2002) reported climatic variations have significant influence on cambial rhythm.

The number of latex vessel rings is a clonal character (Sanderson and Sutcliffe, 1921; Vischer, 1921, 1922 and Bobilioff, 1923) and the frequency of laticiter differentiation is genetically controlled (Premakumari et al., 1992). Depending upon the clone, age of the tree, growth rate, seasonal factors etc. the number of latex vessels varies. Age of the clone is one of
the major factors that determine the frequency of distribution of latex vessel rows in virgin bark. Gomez (1982) reported that in the trees below five years, majority of the laticifer rings were concentrated in the first 4 - 5 mm, and only 40% being in the second. Between five and ten years, laticifer rings were concentrated near the cambium and it would be almost zero near the eight millimeter and by about 25 years about 75% of the latex vessel rings were oriented at the inner most five millimeter of the bark. Bark is regenerated due to the continued activity of vascular cambium. During this process new phloem tissues are produced and normal process of laticifer differentiation continues. The protective tissue lost by tapping is replaced by the formation and activity of a new phellogen below the cut surface (Bobilioff, 1923; Panikkar, 1974).

Various workers have already established highly significant correlations between yield and structural features. Bobilioff (1920), La Rue (1921) and Taylor (1926) reported significant correlations between yield and number of latex vessel rows in seedling progenies. Elaborate studies and yield component analysis proved that the number of latex vessel rows is the major single factor related to yield. When this character was related with girth and plugging index, that accounted for 75 per cent of the yield variations in young plants. A significant correlation between number of latex vessel rows and initial flow rate could be identified (Sethuraj et al., 1974b). Laticifer area and orientation of latex vessel rows are reported to be the factors that influence yield of Hevea clones (Premakumari et al., (1988).

2.3. Genetic studies on yield and major yield components

2.3.1. Variability, heritability and genetic advance

The success of any breeding programme usually depends upon the quantum of genetic variability present in the materials. The knowledge of genetic variability, heritability
and genetic advance in *Hevea* is very essential for a breeder to choose desirable parents and to decide the correct breeding methodology for crop improvement. Genetic studies in *H. brasiliensis* are time consuming, mainly due to the perennial habit of the planting materials. Simmonds (1969) reported that most of the differences between family yields could be accounted for by additive gene effect. Analysis of variance were carried out on yield and girth of seedling progenies of earlier hand pollination (Gilbert *et al.*, 1973; Nga and Subramanian, 1974; Tan *et al.*, 1975; Tan, 1975; Alika, 1980 and Liang *et al.*, 1980). In analysis of variance, magnitudes of sum of squares of relevant terms as well as variance components are used to quantify sources of variation.

The economically important characters like yield and components of yield are not determined by a single gene but it is polygenically controlled. To ascertain the influence of genes and various nongenic factors, detailed biometrical study is essential. However this is relatively limited. The knowledge of the phenotypic variance of a trait and its separation into genetic and environmental components is useful for helping breeders to design an effective selection method. Genotypic coefficient of variation indicates the relative magnitude of genetic diversity present in the material and helps to compare the genetic variability present for different characters. Mydin (1992) reported high genetic variability for volume of latex under stress, plugging index under stress, annual mean dry rubber yield, and dry rubber yield during stress and peak periods was indicated by high estimates of genotypic coefficient of variation. Markose (1984) reported high genotypic coefficient of variation for dry rubber yield, latex volume, bark thickness and latex vessel rows, however, DRC showed low GCV. Licy *et al.* (1992) indicated high GCV for total volume of latex and lowest for bark thickness, among a set of 23 hybrid clones. Licy (1997) reported that the phenotypic
The coefficient of variation was higher than genotypic coefficient of variation for all the characters studied. However, it was closer for most of the characters suggesting less environmental influence. Moderate to high GCV is observed for the annual mean dry rubber yield, summer yield, peak yield, volume of latex, girth increment rate, number of latex vessel rows in virgin, and renewed bark, rate of latex flow and plugging index.

Heritability is useful for comparing and improving the efficiency of selection methods. It is mathematically defined as the ratio between the additive variance ($\sigma^2A$) and the phenotypic variance ($\sigma^2P$). Varying levels of heritability for yield and major yield components has already been reported (Liang et al., 1980; Alika, 1982; Markose, 1984; Mydin, 1992 and Licy, 1997). It estimates the degree of resemblance between offsprings and parents. Heritability decreases with the increase in environmental component of variance for the character under selection (Varghese, 1992). The major functions of heritability estimates are to provide information on transmission of character from the parent to the progeny. Such estimates facilitate evaluation of hereditary and environmental effects in phenotypic variation and thus aid in selection. Heritability estimate can be used to predict genetic advance under selection so that breeder can anticipate improvement from different types and intensities of selection. Information in advanced generations on estimates of heritability and genetic advance on rubber yield and its components in Hevea is very limited (Simmonds, 1986a). Genetic advance under selection can be estimated from the given heritability value (Alika, 1982; Simmonds, 1989). Tan and Subramanian (1976) established additive inheritance for several seedling characters in the nursery. Gilbert et al., (1973) and Nga and Subramanian (1974) noted that yield and girth variation can be largely accounted for by additive genetic variation.
Markose (1984) reported that broad sense heritability was high for dry rubber yield, latex volume, bark thickness and number of latex vessel rows. Mydin (1992) reported that additive gene effects offering scope for improvement through selection was indicated for dry rubber yield, latex flow rate and volume of latex girth increment rate, annual plugging index, plugging index under stress by the moderate to high heritability estimates along with high genetic advance for these traits. Non-additive gene action was indicated by the high heritability and low genetic advance for dry rubber content during the three periods, girth and bark thickness. Licy et al., (1992), reported that nature and magnitude of genetic variability, heritability and heterosis were assessed in 23 F1 hybrid clones, derived from the cross between RRII 105 and RRIC 100 of Hevea brasiliensis at premature phase. Mean annual yield exhibited a high heritability with high genetic advance. Licy (1997) reported high heritability coupled with high genetic advance observed for some of the economic traits like dry rubber yield, volume of latex, rate of flow.

2.3.2. Genetic divergence

Multivariate analysis by means of Mahalanobis D\(^2\) statistic has been recognized as a powerful tool in the hands of breeders to quantify the degree of divergence between genotypes, biological population at genotypic level.

Based on D\(^2\) analysis for yield and various yield components a set of mature Wickham clones were grouped in to eight (Markose, 1984; Mydin et al., 1992) and nine (Abraham et al., 1997) genetically divergent clusters. Considerable genetic diversity was revealed by the wide range of D\(^2\) values and intra and inter cluster distances. The forty clones were grouped into genetically divergent clusters irrespective of their country of
origin indicating the absence of any relationship between geographic diversity and genetic divergence (Markose, 1984; Mydin et al., 1992).

2.3.3. Association of characters

The component traits are not independent in their action but are interlinked and in this complex genetic system, selection practiced for an individual trait might subsequently bring about a simultaneous change in the other. Thus an understanding of the association among component trait is essential to bring a rational improvement. Breeders regularly have to improve two or more traits simultaneously, such as high yield with desirable secondary attributes. Such traits often show correlated response.

A knowledge of the association of quantitative traits, especially of yield and its attributes will be of immense practical value in crop breeding programme. Selection pressure can be profitably exerted on any of these easily discernible characters having close association with yield (Kamalam et al., 1978). Correlation studies between various yield components at nursery stage have established the influence of vigour, bark thickness and number of latex vessel rows on the yield of Hevea clones (Ho et al., 1973; Narayanan and Ho, 1973).

Grantham (1925) and Heusser and Holder (1931) found a negative correlation between yield and dry rubber content. Lee and Tan (1979) found a close association between daily latex volume and yield of rubber and suggested that latex volume was a dominant factor determining yield. Correlations between yield and morphological characteristics of the planting materials have been attempted by various workers (Whitby, 1919; Sanderson and Sutcliffe, 1929; Dijkman and Ostendorf, 1929; Gilbert et al., 1973; Narayanan et al., 1973; Lee and Tan, 1979; Liang et al., 1980; Liu, 1980; Filho et al., 1982; Hamazah and Gomez, 1982 and Pavia et al., 1982). Licy (1997) reported that genotypic correlation in general was higher than
phenotypic correlations in most of the cases, among the 18 characters studied, most of the correlations were found to be in the positive direction. At both phenotypic and genotypic level, summer yield, peak yield, volume of latex, number of latex vessel rows in both virgin and renewed bark and virgin bark thickness exhibited high positive association with annual mean dry rubber yield. The above characters in turn showed high positive association among themselves too. This suggests the scope for simultaneous improvement of these traits by selection, which in turn will improve yield as well. A positive correlation between initial flow rate of latex and number of latex vessel rows in a population of cross-pollinated families was estimated (Sethuraj et al., 1984).

From the correlation studies it has been reported that yield and girth are related to each other and in general positively correlated (Narayanan and Ho, 1973; Liu, 1980). Significant positive correlations of dry rubber yield with volume of latex, latex vessel rows and virgin bark thickness have been reported (Wycherley, 1969; Narayanan et al., 1974 and Markose, 1984). A negative correlation of girth increment with rubber yield has been observed (Narayanan et al., 1973) for trees under tapping where the plant assimilates are partitioned in favour of latex formation rather than growth, particularly in case of clone having high yield potential.

Markose (1984) and Mydin (1992) reported that yield was positively and significantly correlated with latex volume, bark thickness, and number of latex vessel rows. The correlations of bark thickness and latex vessel rows with yield were found mediated through volume of latex at both genotypic and phenotypic levels, annual mean dry rubber yield showed moderate to high positive correlation with dry rubber yield during the stress and peak periods, volume of latex, dry rubber content and latex flow rate during the various
seasons, girth, girth increment rate, length of tapping panel and bark thickness and negative correlations with yield depression under stress and plugging index during three periods.

2.4. Latex and rubber properties

The chemical composition of freshly tapped *Hevea* latex is complex compared to synthetic lattices. This is because *Hevea* latex is a cytoplasm. In addition to the rubber hydrocarbon *Hevea* latex contains a large number of non-rubber constitutes in small quantities. Many of these are dissolved in the aqueous serum of the latex, others are adsorbed at the surface of the rubber particles and some are the non-rubber particles suspended in the latex. A typical composition of fresh latex is as follows.

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Solid Content</td>
<td>41.5%</td>
</tr>
<tr>
<td>Dry Rubber Content</td>
<td>36%</td>
</tr>
<tr>
<td>Protein</td>
<td>1.4%</td>
</tr>
<tr>
<td>Neutral lipids</td>
<td>1.0%</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>0.6%</td>
</tr>
<tr>
<td>Ash</td>
<td>0.5%</td>
</tr>
<tr>
<td>Inositol and Carbohydrates</td>
<td>1.6%</td>
</tr>
<tr>
<td>Other Nitrogen compounds</td>
<td>0.3%</td>
</tr>
<tr>
<td>Water</td>
<td>58.5%</td>
</tr>
</tbody>
</table>

There are 3 major particular components suspended in an ambient serum. They are the rubber particles, lutoids and the Frey-Wyssling particles. Rubber particles constitute 25 - 45% of the volume of the latex in fresh latex. The rubber particles in fresh latex are protected by a complex film containing proteins and lipids. The rubber, contained in the particles, is non water - soluble and occurs as molecular aggregates.
Natural rubber latex is a milky liquid that consists of extremely small particles of rubber suspended or dispersed in an aqueous medium. It is obtained from a great variety of plants, but *Hevea brasiliensis* is the only commercial source of latex. The chemical structure of natural rubber is cis-1,4-polyisoprene, it is a high molecular weight polymer. In addition to the pure rubber hydrocarbon, natural rubber contains various other substances like proteins, fats and fatty acids carbohydrates, mineral matter etc. Allen and Bloomfield (1963) reported the hydrocarbon content is about 94%. The presence of non-rubber substances, though they are in small concentrations, is reported to influence the chemical and physical properties of the hydrocarbon polymer. The properties of natural rubber depend upon the state of cross-linking. It has reported that the cis content of the polymer in NR is to be almost 100 per cent. However Tanaka, (1985) reported the presence of about three trans unit per chain.

The ultimate objective of *Hevea* breeding is to improve the yield potential and economically important secondary characters. But selections of genotypes having latex with good technological properties are also important in present scenario. Some technological characters of rubber such as it's viscosity or plasticity retention index (PRI) could be a selection target if improvement were deemed economically (Demange *et al.*, 2001). At present the selection is based mainly on biological characteristics such as yield of latex, girth and resistance to disease and wind damage. However a high yielding clone with vigorous growth and resistance to maladies need not always produce latex and rubber of desirable properties. A study of the properties of the clonal rubbers is of importance because proper understanding of these characters will enable plant breeders to consider related properties.
in their choice of clones for planting. More emphasis is now being placed on the properties of the latex and rubber obtained from individual clones. Fuller (1988) reported that a major source of variability within and between natural rubber grades is probably the difference in the property of the latex derived from different clones. Several properties of clonal lattices have been studied and documented (Subramanian, 1975). The physical properties of clonal rubber have been less examined. Different clones have different characteristics and give rubber with different properties. The colour and composition of the latex and the plasticity of the rubber tend to be uniform within a clone and different for different clones (Martin, 1961). Seasonal factors and soil characteristics could also affect both the quantity and composition of the latex (Ebi and Kolawole, 1992). Reports on, systematic study of lattices and rubber properties of different clones are scanty.

2.5. Molecular markers

Molecular markers have a great potential for plant breeding as it promises to expedite the time taken to produce crop varieties with desirable characters. The efficiency of conventional plant breeding is greatly improved, as the selection is based not directly on the trait but on molecular markers linked to that trait. In Hevea, studies on the application of molecular markers have been initiated and a few reports on the potential use of this powerful technique are available. Molecular markers like isozyme have been utilized for various purposes in plant breeding.

Isozymes are multiple forms of an enzyme that differed by minor variations in amino acid composition reaction, but exhibit different physical or kinetic properties. Bergman (1987); Cousineau and Donnelly (1992); Granger et al., (1992) reported that isozymes can provide useful information at the genomic level and are widely used to identify and discriminate
cultivars in many agricultural and horticultural crop species. Sreelatha et al., (1993) utilized isozyme polymorphism for the identification of different cytotypes of *Hevea brasiliensis*.

The characterization and identification can be carried out on the basis of phenotypic differences of electrophoretic banding patterns. The advantage of isozymes is that these patterns can usually be interpreted in terms of loci and alleles. The phenotypic approach for characterizing and comparing populations has been criticized because it does not provide genome information (Crawford, 1983; Simpson and Withers, 1986). Therefore, isozymes are ideal genetic markers when estimating genetic variability and plant population. The advantages of isozymes over other biochemical markers are (a) allelic expression is generally co-dominant, free of epistatic interaction and usually unchanged by environmental effects (b) alleles of different loci are generally distinguishable (c) enzymatic systems to be studied are usually chosen for technical reasons independent of their level of genetic variability, as a result of this they can represent a random sample of the genome (d) allelic differences are always detected as mobility difference independent of the functioning and level of variability of each enzyme system.

Genetic diversity among wild and cultivated populations of *Hevea* was assessed using isozymes (Yeang et al., 1998). Isozymes can be utilized for the identification of cultivars, (Yeet et al., 1977), provide information on the organisation of gene pool in a large number of species (Gottlieb, 1981). Isozyme markers are utilized in *Hevea* breeding programmes such as the assessment of genetic diversity and relatedness (Chevallier et al., 1985; Chevallier, 1988; Besse et al., 1994) estimation of outcrossing rates (Sunderasan et al., 1994) and clone identification (Leconte et al., 1994).
The potential use of isozymes in tree breeding programme (Adams, 1983) has not been exploited. This technique is used for the conformity of the planting materials in the bud wood gardens (Yeang, 1988; Leconate et al., 1994). Isozymes offer most reliable single gene markers and they are often codominant in inheritance (Arulsekar and Parfitt, 1986).

Tanksley and Orton (1983), Nielsen (1984), Simpson and Withers (1986), Kahler and Price (1987), Chapman (1989), Hamrick and Godt (1990) etc. reviewed plant isozymes dealing with crop population, genetic variability and characterization. In addition to the characterization and identification of cultivars varieties and natural population, isozyme electrophoresis is used for (a) varietal uniformity assessment (Arus, 1983) (b) phylogenetic studies (Crawford, 1983; Simpson and Withers, 1986) (c) estimation of mating system and selection parameters (Tanksley and Orton, 1983; Brown et al., 1990) (d) evaluation of seasonal variation (Evans and Sharp, 1986) and (e) genetic linkage mapping (Tanksley, 1983; Allard, 1990 and 1999).

Though isozymes are the powerful tool for the characterization and estimation of genetic divergence, they have certain limitations in plant breeding. The two major drawbacks in using isozymes and protein as markers are (1) not all the genetic changes occurring at the DNA level are detected at the protein level (2) only one set of structural genes of organisation are represented in these proteins and this set may not be representative of the whole genome. Another limiting characteristic of isozyme systems in population studies is the relative low number, which can be observed by gel electrophorsis. Of about 3000 enzymes known in plants, only about 60 have been analyzed for isozyme polymorphism (Vellejos, 1983) listed 57 different isozyme system. Isozymes are also influenced by environment and development.
DNA markers are considered to be superior to examine the genetic relationship between clones / cultivars because of the availability of a larger number of potential polymorphic sequences. These markers do not depend on environment and development. These include restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR) and random amplified polymorphic DNA (RAPD). These technique differ in their principles and generate varying amounts of information (Das et al., 1999). Detection of DNA polymorphism by PCR based RAPD gained more importance due to its simplicity, efficiency, relatively easy to perform and non-requirement of prior DNA sequence information (Venkatachalram et al., 2002). Recently the applicability of RAPD markers for genetic analysis in Hevea was evaluated in a set of 24 clones from the breeding pool of RRII (Varghese et al., 1997). Among different clones selected RRIC 100 displayed the highest mean genetic distance. Use of this clone as a parent in hybridization programmes has resulted in highly heterotic hybrids (Licy et al., 1996).

Application of molecular tools in rubber tree improvement is lagging behind because of limited knowledge of the genome. The genetics of the rubber tree has been poorly investigated (Lespinasse et al., 2000). In Hevea the long juvenile period would make RAPD markers an extremely useful tool for identification of cultivars during propagation and planting (Varghese et al., 1997).

A variety of molecular techniques have been used to study the extent of the genetic variation between different wild and cultivated Hevea clones. Among the different techniques, isozymes and RFLP were used for the assessment of genetic variability between wild and cultivated population (Chevellier, 1988 and Besse et al., 1994) and RFLP was used to estimate phylogenetic relationship from mitochondrial DNA (Luo et al., 1995) and to assess the genetic variability from ribosomal DNA (Besse et al., 1993). Genes for powdery mildew
resistance was identified by the RAPD analysis (Shoucai et al., 1994). Varghese et al., (1997) also reported that DNA polymorphism could be detected within 24 Hevea clones. Recently, Lespinesse et al., (2000) established the first genetic map of Hevea brasiliensis using RFLP, ALFP, microsatellite & isozyme markers. The results of DNA polymorphism in 37 cultivated Hevea clones using RAPD analysis involving 80 random oligonucleotide primers are reported by Venkatachalam et al., (2002). The observed polymorphism may be useful for developing molecular markers helpful for screening various traits in Hevea improvement programmes.

Even though morphological traits are also commonly used to determine genetic relationships, they do not provide good estimates of genetic distance because they are influenced by environment and are not variable enough to adequately characterize genetic differences among elite genotypes.

2.6. Progeny analysis

The propagation of Hevea brasiliensis is carried out using seeds or vegetative parts. Earlier the propagation of the crop was through seeds only. However, vegetative propagation using buds became common in later years (Marattukalam et al., 2000). At present seeds are used mainly for producing rootstocks. Special types of seeds known as polyclonal seeds are used directly for propagation. Polyclonal seeds are hybrid seeds that are produced in polyclonal seed gardens. Here several clones are planted intermixed to maximize optimum cross-pollination. The clones planted in these gardens should possess desirable characters like high yield, disease resistance, vigour, ability to produce good seedling families and profuse production of seeds. The number of clones in the seed gardens usually varies from three to seven (Simmonds, 1986b). To maximize cross-pollination, special designs are adopted while planting (Marattukalam et al., 2000). Superior clones are used as
parents in open-pollinating seed gardens in order to produce superior seedling progeny. The technique remains in use today. Saraswathyamma (1990) reported the evaluation of seedling progenies of male sterile clones in *Hevea* based on juvenile yield and secondary attributes.

Polyclonal seedlings resultant of cross pollination express heterosis or hybrid vigour. Polycross or synthetic seedling populations of polyclonal seed gardens of good clones have been successfully used as planting materials. Allogamy coupled with seed propagation increases variation through genetic recombination in seedling population. The seedling population have special agricultural merits in maintaining the genetic variability and adaptability of the population (Mydin, 1990; Varghese, 1992). Seedlings, though not comparable with high yielding clones in production potential, have a special agricultural merit for raising superior polycross progeny from special polyclonal seed gardens. The evaluation of such polycross population can be considered as selective breeding. Such ‘multi parent’ first generation synthetic varieties have been economically successful for many decades, predominantly due to additive genetic control of vigour and yield as well as high general combining ability (Simmonds, 1986b; Tan, 1987).

The genetic superiority of selected mother trees can be identified through progeny testing. The estimation of genetic superiority of mother parents through seedling progeny analysis is also referred as prepotency testing. Allard (1960) reported that prepotency is the capacity of a parent to impress characteristics on its offsprings so that they resemble that parent and each other more closely than usual where gene combination tend to cohere but do not recombine resulting in some sort of functional homozygosity (Harland, 1957). In cashew the study was carried out to estimate large number of cashew mother trees in
relation to the characters of their seedling progenies to formulate an efficient method of 
evaluation of mother trees for large scale production of quality seeds (George et al., 1984). 
However in coconut palm, a study was undertaken to formulate an efficient method for 
evaluation of genetic superiority of mother plants through early seedling progeny analysis 
(Shylaraj and Gopakumar, 1987). Mydin (1990) reported that the prepotent ability of a clone 
to produce high quality seedlings could be determined by systematic and planned 
experiments like seedling progeny analysis. Mydin et al., (1996) indicated that high mean 
performance of the progeny of a clone coupled with high proportion of superior seedlings 
within the progeny is indicative of the ability of the parent to transmit superior traits to its 
progenies. Based on the high performance index and high recovery of superior seedlings 
of certain clones were identified as likely prepotents. Mydin et al., (2002) reported prepotent 
clones for use as seed garden components, based on half-sib progeny analysis of eleven 
clones recommended for planting in India. The two year old progenies showed significant 
variation for test tap yield, girth and bark thickness. Superior progenies were identified by 
performance index based on juvenile traits, the recovery of superior seedlings within each 
progeny was worked out. Out of the eleven clones evaluated five clones were identified as 
likely prepotent with high performance index and high recovery of superior and elite 
seedlings in their progeny.

Studies have indicated the scope for identification of likely prepotents on the basis 
of a performance index, computed for the seedling progenies of clones (Mydin et al., 1990). 
Nine clones identified as likely prepotents on the basis of seedling progeny analysis at the 
age of two years (Mydin, 1992). Markose (1984) reported that no significant difference was 
noted in early growth behavior of clonal seedling progenies raised from open pollinated
seeds at 10 months growth in nursery. It appears that the expression of clonal characters in seedlings needs further growth in field.

2.7. Recent trends in *Hevea* breeding

In India, more than eighty per cent of the rubber plantation is being planted with a single clone i.e., RRII 105, developed by the Rubber Research Institute of India. The major reason for this is due to its yield potential. This clone is also occupying more than ninety percent of the new planting area. This practice has led to a situation of monoclonal planting for the last two decades. If this trend continues it can lead to serious consequences like disease epidemics common to such monoculture plantations. So as a precaution, Rubber Board recommended multiclonal planting to prevent epidemics or other damage vulnerable to such monoclonal cultivation. Liyanage *et al.*, (1989) reported that severe incidence of *Corynespora* leaf disease affected RRIC 103 in Sri Lanka leading to its withdrawal from the planting recommendations. RRII 105 has been reported to be infected by this disease in Karnataka region of India (Jacob, 1997). Even though, there is no alarming situation at present, there is a need for planning alternate measures to prevent possible danger (Saraswathyamma *et al.*, 2000). In order to enrich the genepool for utilization in hybridization programmes, RRII had introduced *Hevea* clones from other rubber growing countries. Saraswathyamma *et al.*, (1992 and 2000) reported that a total of 127 clones have been introduced in to India from other countries. India has obtained clones developed in China, Indonesia, Ivory Coast, Liberia, Malaysia, South America, Sri Lanka and Thailand. Clones developed in India were supplied to China, Ivory Coast, Malaysia, Sri Lanka and Thailand (Saraswathyamma and Maratattukalam, 1996; Saraswathyamma *et al.*, 2000). The recent approach in *Hevea* breeding is to develop not only a high yielding clone but clones having
other desirable secondary attributes along with yield. RRIM 928, RRIM 929, RRIM 931, RRIM 2014 etc. are some of the latex timber clones developed by Rubber Research Institute of Malaysia. In India RR II 5 and RR II 203 are noted for their average yield and good quantity of timber (Saraswathyamma et al., 2000). As per the latest information the present clones are classified in various categories based on the performance (IRRDB, 2001). In Malaysia PB 280, PB 260 and PB 217 are included in category I, PB 280 considered as latex-timber clone. PB 255 in category II whereas, PB 310, PB 311, PB 312 and PB 314 in category III. In Vietnam PB 255 as class I clones, however in Indonesia PB 217 and PB 260 as latex clones in class I category. The yield performance of the clone PB 255 and PB 260 are high in Thailand as these clones were recommended as class I clone and PB 235 as class II clone. In Sri Lanka PB 217 is classified as group I and PB 235 and PB 260 in class II, the clone PB 255 is in third group. In Cambodia, PB 235 was included in class I and PB 217 and PB 310 in class II clones, however PB 255 and PB 260 are class III category. The yield performance of PB 217 in Cote d’ Ivorie was high hence it included under class I, PB 235 and PB 260 in class II whereas, PB 255 in class III category.

Improving the quality of latex is also important as natural rubber forms the raw material for various industrial and technological purposes. Hence the study related to quality improvement of natural rubber of Hevea clones is also worthwhile. Synthesis of clones having combined effect of high production potential with excellent latex and rubber properties should prove to be rewarding. Molecular markers are importance in perennial crops like Hevea where the conventional genetic analysis is difficult due to long breeding and selection cycle and difficulties in raising next generation. Isozymes and DNA based RAPD markers are effective tools for analyzing the genetic relationship between clones.
The application of RAPD markers for genetic analysis was evaluated in a set of clones from the breeding pool of RRRII. Among different clones, one which recorded the highest genetic distance, are utilized as parent in hybridization programme that resulted in highly heterotic hybrids. Characterization of latex and rubber properties is another trust area, which should be considered in breeding programme to improve the quality of raw rubber that extracted from the clones of *Hevea*. Different clones have different characteristics and give rubber with different properties.